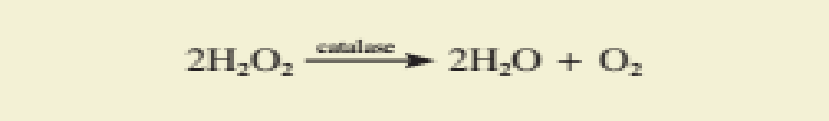
PW 01 : BIOCHEMICAL TESTS

CATALASE and OXYDASE

1. CATALASE TEST

1.1. Introduction

When aerobic bacteria grow by respiration, they use oxygen as a terminal electron acceptor, converting it to water. However, they also produce hydrogen peroxide as a by-product of this reaction. Hydrogen peroxide is a highly reactive oxidizing agent that can damage enzymes, nucleic acids, and other essential molecules in the bacterial cell. To avoid this damage, aerobes produce the enzyme ***catalase***, which degrades hydrogen peroxide into harmless oxygen and water.



Strict anaerobes and aerotolerant bacteria such as *Streptococcus* lack this enzyme, and hence they are unable to deal with the hydrogen peroxide produced in aerobic environments. The presence of catalase is one way to differentiate these bacteria from aerobes or facultative aerobes, both of which produce catalase. For example, catalase production can be used to differentiate aerobic staphylococci (*Staphylococcus)* which is Gram positive from streptococci (*Streptococcus)* and enterococci *(Enterococcus)*, which is Gram positive and lack this enzyme but produce *superoxide dismutase* and *peroxidase*.

1.2. Test principal

To determine if catalase is produced, a small amount of growth is transferred to a clean microscope slide and mixed with 2 drops of 3% hydrogen peroxide. If catalase is produced, there will be vigorous bubbling due to the breakdown of hydrogen peroxide (H_2O_2) and the production of water (H_2O) and oxygen gas (O_2) (Fig. 01).

1.3. Material

- 3% hydrogen peroxide
- Petri dishes contain the bacterial strains to be studied: *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Bacillus sp*.

Figure 01: Result Interpretation of Catalase Test (**Source:** microbiology info.com).

1.4. Procedure

1. Using the end of a wooden swab, transfer some cells from the *S. aureus* culture to the surface of a clean microscope slide.

2. Add 2 to 3 drops of 3% hydrogen peroxide to the cells, mix with the wooden stick, and observe for vigorous bubbling (The bubbles are the molecular oxygen that is produced as the *catalase* breaks down the hydrogen peroxide).

- *Catalase-positive:* bubbles observed.
- *Catalase-negative:* no bubbles.

3. Repeat the same procedure for your test organism and record your results.

1.5 Results

Organism: _____ Catalase Reaction: + or – *(circle one)*

Organism: _____ Catalase Reaction: + or – *(circle one)*

1.6 Study questions

1. Why is it important to use fresh bacterial cultures for the catalase test?

2. How do you interpret a negative result in the catalase test?

3. How would you perform a catalase test on a bacterial colony isolated from a blood agar plate?

4. What factors could cause a false negative result in the catalase test?

5. In attempting to distinguish between *Staphylococcus* and *Streptococcus* species, what is the ***first*** lab test that should be performed? Why?

6. *Streptococcus* and *Enterococcus* species do not produce the enzyme *catalase*. How are these aerotolerant bacteria able to survive in the presence of oxygen when they lack this important enzyme?

2. OXYDASE TEST

2.1. Introduction

The oxidase test detects the presence of a ***cytochrome oxidase*** system that will catalyze the transport of electrons between electron donors in the bacteria and electron acceptors (cytochrome oxidase is the last enzyme in the respiratory chain that ensures the transfer of electrons to oxygen or another mineral oxidant). This test is typically used to differentiate between **oxidase-positive** bacteria like *Pseudomonas, Neisseria, Alcaligens, Aeromonas, Campylobacter, Vibrio, Brucella* and *Pasteurella* and **oxidase-negative** bacteria like *Enterobacteriaceae* (*Escherichia coli*).

2.2. Principle

Cytochrome containing organisms produce an intracellular ***oxidase enzyme***. This oxidase enzyme catalyzes the oxidation of cytochrome *c*.

Phénylène diamine oxydase refers to an enzyme that catalyzes the oxidation of **phenylenediamine**. The enzyme promotes the transfer of electrons from phenylenediamine to an electron acceptor, typically oxygen, which results in the oxidation of phenylenediamine and often a visible color change.

In the context of microbiological testing, **phénylène diamine oxydase** can refer to enzymes involved in the **oxidase test**, which detect the presence of cytochrome *c* oxidase in certain bacteria. This enzyme reaction is often used for bacterial identification, where the oxidation of phenylenediamine produces a color change (typically blue or purple) as a positive result for oxidase activity.

2.3. Material

- A disk pre-impregnated with the reagent N, N, N', N'-tetramethyl-*p*-phenylenediamine dihydrochloride.
- Petri dishes contain the bacterial strains to be studied: *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Bacillus sp*.

1.4. Procedure

1. Tack the disc pre-impregnated with the reagent and crush a colony of germs to be studied on this paper with the tip of a Pasteur pipette (instrument that does not oxidize the reagent).

2. A positive reaction is indicated by an intense deep-purple hue, appearing within 5-10 seconds, a "delayed positive" reaction by coloration in 10-60 seconds, and a negative reaction by absence of coloration or by coloration later than 60 seconds.

3. Repeat the same procedure for your test organism and record your results.

1.5. Results

- Positive Result: Development of a deep purple-blue/blue color indicates oxidase production within 5-10 seconds.
- Negative Result: No purple-blue color/No color change.

1.6. Study questions

1. How does the presence of cytochrome *c* oxidase in bacteria affect the result of the oxidase test?

2. What is the role of the reagent tetramethyl-*p*-phenylenediamine in the oxidase test?

3. Can the oxidase test be used to identify both aerobic and anaerobic bacteria? Why?

4. Why is it important to perform the oxidase test on a fresh bacterial culture?

5. What are the limitations of the oxidase test in bacterial identification?

6. How would you interpret the results of an oxidase test if a bacterium shows weak or delayed color change?